SERT a 12-helix presynaptic plasma membrane protein terminates synaptic transmission by Na+ symport of the neurotransmitter serotonin (5-hydroxytryptamine, 5-HT) from the extracellular mileu of synapse into the cell.
Crystal structures of Leucine transporter (LeuT), the bacterial homologue of SERT, represent a substrate-bound occluded conformation in which the the intracellular part of the transporter is closed and packed.
We have constructed an experimentally-validated homology model of SERT based on the LeuT structure and cognate information about other transporters in this family and performed SMD simulations by pulling serotonin from the primary binding site towards the cytoplasm, to explore the alternative inward-facing conformation and reveal the cytoplasmic permeation pathway. The initial state of the SERT model has 5-HT both in the primary binding site and the newly discovered secondary site (L. Shi et al, Mol Cell, 2008). Local structural rearrangements associated with substrate movement reveal changes in water solvent accessibility that explain the energetic drive of the transport mechanism and provide data for validation against experimentally determined accessibilities measured with the substituted cysteine mutagenesis accessibility method (SCAM). Interactions of the moving substrate in SMD identify the residues of SERT which line the transport pathway and their role in stabilizing the inward-open or inward-closed state of SERT. These states involve large-scale helix movements triggered by changes in the binding sites of the substrate and Na ions, and enabled by solvent stabilized changes in the states of an ionic interaction network at the cytoplasmic end of SERT. While the detailed representation of the process provided by SMD simulations at atomic resolution offers specific new hypotheses for experimental probing of the proposed mechanism, it also reveals new insights and mechanistic aspects that are not achievable in other ways.

354-Pos Board B233
Solvent Effects On Protein Mechanical Stability: A Steered Molecular Dynamics Study
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The solvent is an integral component in all cellular processes and solvent composition is actively regulated in the living cell. Changes in solvent environment constitute a chemical signal that is transduced into a mechanical response: a chemically induced change of mechanical properties of a protein system. In this study, steered molecular dynamics simulations are used to stretch mechanical proteins set in non-aqueous solvent environment to reveal details and mechanism of protein - solvent interactions. We explore the atomic level mechanism and measure the effect of solvent substitution on the mechanical properties of proteins. We investigate the distance to the transition state during force induced unfolding as a function of solvent molecule size and the atomistic detail and timescale of participation of solvent molecules in the transition state structure. Resulting constant velocity and constant force extension profiles showed increased stability and resistance to unfolding force by proteins solvated in deuterium oxide vs. proteins solvated in water. Solvent molecules were found present the transition state structure and involved in forming a stabilizing bridge between the force-bearing sheer topology elements. Features of the simulations were also matched with previously reported experimental results.

355-Pos Board B234
Fluorescence Resonance Energy Transfer Reveals Key Binding Domains of Neurotrophin Receptor-Interacting Melanoma-Associated Antigen Homolog in Bone Morphogenetic Protein-Mediated Apoptosis
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Apoptosis, one form of programmed cell death, is used by tissues to develop normally and maintain homeostasis. Lack of apoptosis underlies many diseases such as cancer, while an excess can cause neurodegenerative disorders. Understanding the molecular events that initiate either condition is necessary for the development of treatments. Bone morphogenetic proteins (BMP) play profound roles in development, such as regulation of neural progenitor apoptosis and glial differentiation. Binding of ligand to the BMP receptors triggers the canonical and non-canonical pathways involving Smad and TAK1 activation respectively. In the non-canonical pathway, p38 mitogen activated kinase ($p38^{MAPK}$) is upregulated in P19 cells, a model line of neural progenitors, triggering a signal cascade leading to apoptosis. X-linked inhibitor of apoptosis protein (XIAP) functions as a positive mediator linking TAK1 to the BMP receptors through TAB1. Neurotrophin receptor-interacting MAGE (NRAGE) homolog binds with XIAP in the XIAP-TAB1-TAK1 complex and is necessary in the non-canonical pathway for apoptosis. We have measured fluorescence resonance energy transfer (FRET) between enhanced green fluorescent protein attached to NRAGE deletion mutants and DsRed-monomer attached to XIAP. Results show that the interaction is direct and is facilitated by a unique tryptophan-glutamine-X-proline-X-X (WQXPXX) repeat in NRAGE. We have continued using FRET with enhanced cyan and yellow fluorescent proteins (FPs) in designs with the FP fused to the amino or carboxy termini of NRAGE deletion mutants and XIAP to compare the FRET efficiencies and verify that the FP placement does not affect the NRAGE-XIAP interaction. Additionally, we compare the FRET of overexpressed NRAGE-XIAP with that of the endogenous interaction using antibodies conjugated to Alexa Fluors.

356-Pos Board B235
Conformational Dynamics of Antithrombin III With its Allosteric Activator Heparin
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Antithrombin III (ATIII) is a serpin that is involved in the regulation of blood coagulation through the inhibition of blood clotting enzymes. Heparin is an allosteric activator of ATIII that binds to helix-D and causes a conformational change in the reactive center loop (RCL), expelling it from its position partially inserted into beta-sheet A. RCL expulsion in turn increases ATIII activity toward fXa several hundred-fold. Hydrogen/deuterium exchange and mass spectrometry were used to probe the dynamics of ATIII in the presence and absence of a synthetic heparin pentasaccharide (Fondaparinux). Results of our initial hydrogen/deuterium exchange mass spectrometry experiments provide direct, solution phase evidence that heparin cofactor binding alters conformational dynamics in four specific regions of the antithrombin molecule. (1) Helix D – Heparin binding reduced H/D, consistent with hD extension upon cofactor binding. (2) Breach region – Beta strands 3A and 5A, flanking the site for RCL insertion into sheet A, showed reduced H/D exchange, consistent with increased rigidity of the breach region and stabilization of a loop-expelled form. (3) Proximal RCL and hinge region – H/D exchange for residues 376-387, which includes the N-terminal hinge region of the RCL, increased in the presence of heparin, indicating greater solvent exposure and expulsion from beta sheet A. (4) Distal RCL – Deuterium exchange for 388-402, which includes s1C and the distal side of the RCL, decreased significantly in the presence of heparin, suggesting that s1C extension-mediated stabilization on the C-terminal side of RCL contributes to exposure of the proximal its end upon cofactor binding. Thus, dynamic H/D exchange studies of free and heparin-bound antithrombin molecules in solution will be useful for validating and refining models of ATIII heparin activation inferred from crystal structures.

357-Pos Board B236
Structural Evaluation of the Effects of Disulfide Bond Eliminations on Scorpion Toxin κ-Hefutoxin1 from *Heterometrus Fulvipes*
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κ-Hefutoxin1, a novel weak potassium-ion-channel toxin present in the venom of the scorpion *Heterometrus fulvipes*, is a 22-residue peptide which has a unique spatial fold consisting of two parallel helices linked by two disulfide bridges without any β-sheets. In order to evaluate the structural contribution of the disulfide bonds, wild and three mutant forms of κ-Hefutoxin1 were cloned, expressed, purified and structurally analyzed. To do so, synthetic genes encoding wild and three mutant forms of k-Hefutoxin1 were designed using appropriate codons and synthesized using overlapping primers. In the mutant forms, alternative pairs of cystein residues, which participate in the formation of disulfide bonds, were replaced by serine (Mut1: C4S, C22S; Mut2: C8S, C18S; Mut3: C4S, C22S, C8S and C18S). To facilitate cloning into expression vector, EcoRI and BamHI restriction sites were inserted to the flanking ends of the genes. Moreover, Tev cleavage site was added to the N-terminal part of the genes. The amplified k-Hefutoxin1 genes of wild and mutant forms were cloned into pET32a vector, followed by transformation into *E.coli* host strain DH5α. The positive colonies with recombinant plasmid were first screened by PCR analysis and finally confirmed by sequencing. Then, the correct recombinant plasmid was transformed into *E.coli* host strain BL21 in which protein expression was induced by IPTG. After assuring protein expression by polyacrylamide gel electrophoresis, the cells containing wild and mutant forms of

k-Hefutoxin1 were lysed by sonication. Obtained proteins were finally purified and structurally analyzed by CD spectroscopy and NMR. In this study, we were able to ascertain the effect of absence of one or both of the disulfide bonds on the structure of k-Hefutoxin1.

358-Pos Board B237

Characterization of HIV-1 Protease-Inhibitor Interaction by Interflap Distance Measurement, NMR Spectroscopy, and Solution Kinetics

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HIV-1 protease (HIV-1 PR) is an important drug target for the treatment of HIV/AIDS. Currently, there are several commercially available protease inhibitors (PIs) that improve the lives of patients. However, viral mutation often renders the PIs less effective after continuous use. In this study, we compare the effects of several PIs such as Indinavir, Atazanavir, Lopinavir, Saquinavir and Nelfinavir on the activity of the wild-type (PMPR), clinical isolate V6 and MDR769 HIV-1 proteases. We also use 2D HSQC NMR of uniformly 15N labeled samples and DEER spectroscopy with K55MTSL as the reporter site to study the conformational change in HIV-1 PR as a function of various inhibitors. Preliminary solution kinetics data show strong inhibition of PMPR by various inhibitors, but not for V6 and MDR769. The NMR spectra of unbound PMPR, V6, and MDR769 differ markedly from one another, and significant changes in the protein chemical shifts of PMPR and V6 are seen in the presence of inhibitors. The DEER distance distribution profiles reveal altered flap conformations in the uninhibited state of V6 and MDR769 compared to PMPR. Finally, in the presence of inhibitors such as Indinavir, the flap conformations in V6 and MDR769 show a minor change, whereas data for PMPR reflects a closing of the flaps to a conformation consistent with X-ray crystallographic structures.

359-Pos Board B238

Picosecond Dynamics Of Surface Water As A Function Of Hydrophobicity

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Previously we and others have shown terahertz sensitivity to protein-ligand binding, possibly arising from the change in low frequency structural modes [1]. Another possibility is that the water adjacent to the protein, which strongly contributes to the THz response, may be affected by the change in the protein surface with binding. Pollack and coworkers have shown an ordered water film (from nm up to hundreds of um thickness) is formed on a smooth hydrophilic surface [2, 3]. To study how picosecond dynamics of water are affected by hydrophilicity of a surface, we performed a series of terahertz dielectric measurements as a function of water film thickness and hydrophicility of the surface. Measurements were made on solution cells with windows made of polyethylene or quartz. The hydrophilicity of the surfaces was modified by air plasma treatments, and characterized with contact angle measurements. Terahertz time domain spectroscopy measurements were made as a function of thickness and the absorption coefficient and index were extracted. The results were analyzed at selected frequencies to study the absorption trend with respect to the change of thickness. These measurements suggest a smaller THz response for water adjacent to hydrophilic surfaces. This lower response may possibly come from an overall decrease in water density at the surface or a stronger ordering inhibiting rotational motions contributing to the picosecond response.

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360-Pos Board B239

The Scaffolding Subunit of PP2A is a Coherent Linear Elastic Object That Can Transmit Mechanical Information Along Its Length

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HEAT repeat protein PR65 is the scaffolding component of protein phosphatase PP2A, which has been implicated in tension sensing during chromosome segregation and in diverse other chromosomal processes. PR65 is composed exclusively of 15 HEAT repeats, i.e. pairs of anti-parallel alpha helices connected by short 1-3 residue turns, that stack in parallel to form a solenoid structure in which the packed helices form one continuous hydrophobic core. Molecular dynamics analysis reveals that tensile or compressive forces applied at the protein termini produce evenly-distributed shape changes (straightening/bending) via longitudinal redistribution of stress, with elastic coherence resulting from the continuous meshwork of van der Waals interactions created by the aligned helix/helix interfaces. At higher forces, fracturing occurs via loss of a specific helix/helix contact between adjacent repeats, accompanied by relaxation that spreads outward from the fracture site through the adjacent regions. Fracturing is nucleated by "flaws" resulting from atypical residues in inter-helix turns along the edges of the structure. Such flaw sites exhibit competition, such that only one of them fractures, as well as cooperation to create bounded regions of increased strain. Thus, PR65 is a coherent linear elastic object, capable of transducing mechanical information from one position along its length to another. We propose that HEAT repeat scaffolds, including PR65, exist to place bound components in mechanical linkage so that their promoted molecular reactions are sensitive to externally-imposed mechanical forces. More generally, since analogous elastic coherence should be present in many types of helical repeat proteins, cells may be filled with mechanically-tunable molecules, and mechanical stress may be a common currency for subcellular information transfer.

361-Pos Board B240

The Closure Mechanism Of M. Tuberculosis Guanylate Kinase Relates Structural Fluctuations To Enzymatic Function

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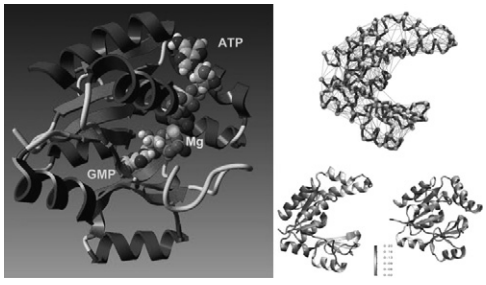
The Allosteric Spring Probe (ASP) technique allowed Zocchi et al. to act on the enzymatic activity of guanylate kinase (GK) by applying tension upon the molecular structure of this enzyme [Choi et al., Biophys. J., 2007]. These experiments raise the question about the underlying conformational modifications leading to such an observation.

In order to elucidate the results from these ASP studies, we investigate the conformational dynamics of GK and its mechanical properties. We use both high resolution atomistic molecular dynamics and low resolution Brownian Dynamics simulations.

The enzyme is subject to large conformational changes, leading from an open to a closed form, and further influenced by substrate or co-factor docking. A reduction or perturbation of the conformational space available to GK can be related to the activity loss encountered in the ASP experiments.

We describe a detailed picture of GK's closure mechanism characterizing the hierarchy and chronology of structural events essential for the enzymatic reaction. Rigidity profiles obtained from simulations of distinct states hint at important differences. We have investigated open vs. closed, apo vs. holo or substrate vs. product-loaded forms of the enzyme.



362-Pos Board B241

The Closed <-> Open Transition of Adenylate Kinase From Crystal Structures and Computer Simulations

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Many proteins function as dynamic molecular machines that cycle between well-defined states. A mechanistic and atomic-scale understanding starts with crystal, NMR or electron microscopy structures in these states. Typically, none or only very limited structural information is available for the intermediates along the transition. Computational methods can simulate transitions between states but due to the absence of intermediate structures it is hard to verify that the simulated transition path is correct. One exception is the enzyme adenylate kinase. It is well studied and a large number of crystal structures are available. Vonrhein et al [1] suggested early on that some of these structures would be transition intermediates due to stabilization by crystal contacts and created a 'movie' from nine structures. We took this idea one step further and compare 45 experimental structures to hundreds of transitions of E. coli AdK simulated with the dynamic importance sampling method (DIMS). We find that DIMS trajectories, which only require a crystal structure for the starting and the end point of the transitions, contain all intermediate crystal structures (RMSD for matches: <4 Å with median 1.2 Å). The crystal structures